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Precision Medicine in Acute Kidney Injury: A Promising Future?

Despite increased focus over the past decade, the management of patients with acute kidney injury (AKI) remains largely supportive, including dialysis for severe cases. Clinical trials in AKI examining timing of dialysis, intensity of dialysis, pharmacotherapy, and novel biologics have been consistently negative (1–6). One postulated reason for this dearth of positive trials is the inherent delay in intervention for patients with AKI due to a reliance on serum creatinine, and researchers have embarked on a decades-long journey to identify a biomarker of AKI that would identify patients while kidney damage was actively ongoing and before serum creatinine increases. Numerous biomarkers of tubular injury have now been identified (7, 8), and in the ICU these biomarkers have modest sensitivity, specificity, and association with outcomes (9, 10). However, these biomarkers have so far failed to recategorize the heterogeneous syndrome of AKI into more clinically useful subtypes or be incorporated into clinical practice. There has been some progress with biomarkers of cell cycle arrest, most notably TIMP2*IGFBP7 (tissue inhibitor of metalloproteinase-2*insulin growth factor binding protein-7), to identify patients at high risk of AKI (11, 12). In patients after cardiac bypass surgery at high risk for AKI (as denoted by elevated TIMP2*IGFBP7), there was a lower incidence and decreased severity of AKI in patients who were randomized to a "KDIGO (Kidney Disease: Improving Global Outcomes) bundle," which included monitoring of hemodynamic parameters, avoidance of nephrotoxins, and holding angiotensin-converting-enzyme inhibitors (13). Although prevention may be possible, the role of biomarkers in guiding treatments or response to therapy remains unclear.

For this reason, the article by Bhatraju and colleagues (pp. 863–872) in this issue of the *Journal* represents meaningful progress (14). The authors applied latent class analysis to a discovery group of 794 patients admitted with systemic inflammatory response syndrome to the ICU and a replication cohort of 425 patients with acute respiratory distress syndrome (ARDS) and identified two subphenotypes of AKI (AKI-SP1 and AKI-SP2). The patients in AKI-SP2 were sicker and had worse renal function; higher rates of sepsis, ARDS, and mortality; and lower rates of renal recovery. The authors determined via least absolute shrinkage and selection operator method that the ratio of angiopoietin-1 and angiopoietin-2 (Ang1/Ang2) and sTNFR-1 (soluble tumor necrosis factor receptor-1) were sufficient to accurately distinguish between the two subphenotypes of AKI (c-statistic > 0.93).

Ang1 and Ang2 are endothelial growth factors, which both bind to the extracellular portion of the Tie-2 receptor. They have opposing actions: Ang-1 stabilizes the vascular endothelium, and Ang-2 destabilizes the vascular endothelium. Consequently, the ratio of these endothelial growth factors provides an assessment of endothelial dysfunction and is associated with prognosis in several cohorts of critically ill patients with and without AKI (15, 16).

These sophisticated statistical techniques and biomarkers determined what clinicians intuitively understand: patients with more severe inflammation do worse. The authors then reidentified the subphenotypes in a random subset of 328 patients from the VASST (Vasopressin in Septic Shock Trial) who had measurements available for Ang1/Ang2 and IL-8 (17). (Soluble tumor necrosis factor receptor-1 was not available in the VASST cohort, but IL-8 was notably different between AKI-SP1 and AKI-SP2 in the discovery and replication cohorts.) This clinical trial was a randomized, double-blind study comparing vasopressin and norepinephrine infusions to norepinephrine alone in 776 patients with septic shock. The study had shown no differences in mortality or rates of renal failure between patients in either treatment group. Once patients were recategorized into the AKI subphenotypes, patients in AKI-SP1 (the less ill group) had improved 90-day mortality with early addition of vasopressin compared with norepinephrine alone. This association persisted

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after adjustment for Acute Physiology and Chronic Health Evaluation II score, suggesting discriminating ability of the AKI subphenotypes beyond simply severity of illness. If replicated, these findings hold the potential to guide clinicians in more accurately assessing prognosis of patients as well as expected response to therapy.

Despite these findings, much work remains. There were no urine specimens available from these cohorts, and it is unclear if the particular model the authors identified is truly the best model to distinguish subphenotypes of AKI in patients with systemic inflammatory response syndrome. Moreover, given the heterogeneity of AKI and the innumerable settings in which it occurs, this particular model may not be helpful for all patients with AKI, such as those post cardiac surgery, post contrast exposure, or with heart failure. Other investigators will have to identify subphenotypes of patients with AKI in these settings, and these subphenotypes may be identified by a combination of biomarkers of tubular injury, cell cycle arrest, cardiac dysfunction, clinical variables, or a novel biomarker. Incorporation of phenotyping may therefore be an important component of future trials for AKI and could possibly be the key to breaking the trend of negative clinical trials in AKI.

It is important to note that our molecular understanding of acute tubular injury, which, if biopsies were available in these cohorts, probably would have been the most common histologic entity, remains suboptimal. Inflammation and endothelial dysfunction are typically considered a systemic response instead of intrinsic to the kidney. The KPMP (Kidney Precision Medicine Project), which will integrate molecular, structural, and clinical information from kidney biopsy specimens of patients with AKI, will be critically important to untangle the molecular underpinnings of acute tubular injury. Ideally, these data will improve our ability to identify disease subgroups that would respond to therapy.

Instead, this particular article can serve as a framework for other investigators to attempt to identify other subphenotypes of AKI. To move forward, we recommend the following next steps. First, investigators should attempt to identify novel subphenotypes of AKI in other common AKI settings using currently existing biomarkers. As our molecular understanding of AKI improves from studies like KPMP, these subphenotypes should ultimately be identified based on underlying molecular mechanisms. Second, investigators should demonstrate that these subphenotypes respond differently to therapies in previously completed clinical trials. This is an important step and, given the plethora of negative clinical trials in AKI, there is ample opportunity. Ultimately, we hope these findings would change the way patients are selected and enrolled into future clinical trials. Although biorepositories require time, money, and effort, we urge investigators to continue maintaining and creating them, as they can yield valuable information years later.

It remains to be proven if the subphenotypes of AKI in patients with critical illness identified by this study will be useful in clinical practice. Regardless, the findings are important because they suggest that it is possible to untangle the complex and heterogeneous clinical syndrome of AKI into groups of patients who respond to a particular therapy. In other words, we may be one step closer to personalized and precision medicine for AKI. ■

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Letting It All Out: Neutrophils in Early Cystic Fibrosis Airway Inflammation

Cystic fibrosis (CF) lung disease is characterized by a vicious cycle of mucus secretion, airway infection, and inflammation. Neutrophils are the primary inflammatory cell involved in this process and are recruited from the blood into the airway lumen early in the disease process, as demonstrated by BAL studies performed in infants with CF (1, 2). These neutrophils contain an array of inflammatory mediators, oxidants, and proteases that are critical for antimicrobial defense. Large amounts of these enzymes escape from neutrophils in cell death and during phagocytosis, and directly damage the airway epithelium. Another mechanism is the release of enzyme through exocytosis, but the mechanisms that control the degranulation and release of these enzymes are less well understood (2). One particular enzyme, neutrophil elastase (NE), is capable of digesting diverse proteins and contributes to the progression of structural lung disease (3, 4). Higher levels of free extracellular NE in sputum have been shown to predict subsequent lung function decline (5). The antiprotease defenses in the airways are designed to neutralize free proteases such as NE and prevent their damaging effects. However, these defenses are eventually overwhelmed and degraded by the protease burden in the lung (6).

In this issue of the *Journal*, Margaroli and colleagues (pp. 873–881) advance our understanding of neutrophilic inflammation in the early CF lung (7). This cross-sectional study included children with CF under 3 years of age with evidence of early changes of CF lung disease as measured by the computed tomography (CT) Perth-Rotterdam annotated grid morphometric analysis for CF (PRAGMA-CF) scoring method, but virtually no bronchiectasis. As expected, BAL fluid (BALF) samples obtained the same day the CT was performed showed neutrophilic inflammation. Findings in the CF cohort were compared with disease control subjects recruited from the Aerodigestive Clinic who were undergoing bronchoscopy for a clinical indication and also showed evidence of neutrophilic inflammation, albeit less marked than that observed in the CF group. The airway neutrophils in individuals with CF demonstrated increased expression of surface markers reflecting hyperexocytosis of NE-rich granules into the airway lumen. This phenotype was seen in the airway neutrophils but not in blood neutrophils, and was not observed in control patients. This suggests that the inflammatory milieu of the CF airway recruits neutrophils from the blood and stimulates them to adopt an activated state. This neutrophil phenotype with hyperactive exocytosis of NE-rich granules correlated with early structural lung damage by CT coupled with the PRAGMA-CF scoring system. Therefore, this distinguishing feature of neutrophilic airway inflammation in CF could potentially be a key process in early CF lung disease.

By contrast, other biomarkers of neutrophilic inflammation, such as the BAL neutrophil percentage and free extracellular NE activity (measured by a sensitive Förster resonance energy transfer–based assay) did not correlate with structural changes on CT. This is contrary to previously reported work of AREST-CF (Australian Respiratory Early Surveillance Team for Cystic Fibrosis) group regarding the role of free NE, which predicted the subsequent development of bronchiectasis (4, 8). Potential reasons for this discrepancy may be the use of a more sensitive assay for free NE in the current study compared with the group's previous studies (4, 8), and the comparison of free NE with the sensitive PRAGMA-CF score, which has been reported to be more sensitive for detecting early structural lung abnormalities (9). In addition, the cross-sectional design of the current study, with structural changes detected at a single time point, may not be reflective of the impact of released NE and the dynamics of airway inflammation over time.

Although the data on neutrophil exocytosis are novel, this study has limitations. First, this was not a specifically designed prospective study; patients were drawn from different cohorts with different inclusion criteria. This may have resulted in a more heterogeneous study population and introduced variability into the data. Second, the control group was small and contained subjects with a mixture of underlying diagnoses. Ideally, individuals with CF would be compared with patients with a disease process also characterized by neutrophilic airway inflammation and structural lung damage over time, such as primary ciliary dyskinesia. Third, clinical data such as the temporal relationship between testing and episodes of increased respiratory symptoms, antibiotic use, respiratory microbiology, or functional measures of lung function (e.g., the lung clearance index) are not available. Fourth, BALF only reflects the inflammatory process the airway lumen and not in the airway wall, where structural remodeling takes place. Finally, surface expression assessed by flow cytometry may not be a direct representation of exocytosis, and a functional assay may be better suited to reflect the impact of exocytosis on the inflammatory process in the airways.

Biomarkers to detect neutrophilic inflammation could be useful for tracking the progression of airway pathology over time and be included as outcome measures in interventional trials. The results of this study raise the question as to whether neutrophil exocytosis could serve as a biomarker for airway inflammation in CF. However, bronchoscopy for BALF is an invasive procedure and impractical for

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